Over-expression of macrophage migration inhibitory factor in human endometrial cancer.

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Gynecologic cancers including cancers of the endometrium are a clinical problem¹⁻⁴. We mined published microarray data^{5,6} to discover genes associated with endometrial cancers by comparing transcriptomes of the normal endometrium and endometrial tumors from humans. We identified macrophage migration inhibitory factor, encoded by MIF, as among the most differentially expressed genes, transcriptome-wide, in cancers of the endometrium. MIF was expressed at significantly higher levels in endometrial tumor tissues as compared to the endometrium. Importantly, in human endometrial cancer, primary tumor expression of MIF was correlated with recurrence-free survival in white patients with low mutational burden. MIF may be a molecule of interest in understanding the etiology or progression of human endometrial cancer.

Keywords: endometrial cancer, gynecologic cancers, endometrium, MIF, macrophage migration inhibitory factor, systems biology of endometrial cancer, targeted therapeutics in endometrial cancer.

Endometrial cancer is the most common gynecologic cancer in the developed world¹. Over the last three decades, the incidence of endometrial cancer has increased 21%⁴ and the death rate has increased 100%³. We harnessed the power of independently published microarray datasets^{5,6} to determine in an unbiased fashion and at the systems-level genes most differentially expressed in endometrial tumors. We report here the differential and increased expression of the macrophage migration inhibitory factor (MIF) in human endometrial cancer.

Methods

We utilized datasets GSE63678⁵ and GSE17025⁶ for this global differential gene expression analysis of human endometrial cancer in conjunction with GEO2R. GSE63678 was generated using Affymetrix Human Genome U133A 2.0 Array technology with $n=5$ control endometrial tissues (including $n=4$ uterine myomas and $n=1$ benign cyst) and $n=7$ endometrial cancers (including $n=2$ endometrial adenocarcinomas, $n=3$ mixed endometrioid adenocarcinomas, and $n=2$ adenocarcinomas with squamous differentiation); analysis was performed using platform GPL571. GSE17025 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with $n=5$ normal endometrial tissues (inactive endometrium) and $n=91$ endometrial cancers; analysis was performed using platform GPL570. The Benjamini and Hochberg method of p -value adjustment was used for ranking of differential expression but raw p -values were used to assess statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether MIF gene expression was significantly different between control endometrial tissue and endometrial tumor tissue in humans using a two-tailed t-test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter tool⁷ for correlation of MIF mRNA expression levels with recurrence-free survival in $n=543$ endometrial cancer patients.

Results

We harnessed the power of blind comparative transcriptome analysis using published microarray data^{5,6} to discover in an unbiased fashion genes associated with endometrial cancer in humans.

MIF is differentially expressed in endometrial cancer.

We identified macrophage migration inhibitory factor, encoded by MIF, as among the genes most differentially expressed in cancers of the endometrium when compared to benign endometrial tissues (Chart 1). When sorting each of the genes expressed in endometrial tumor tissue based on significance of change in expression as compared to benign endometrial tissue, MIF ranked 133 out of 22273 transcripts, equating to 99.4% differential expression (Chart 1). Differential expression of MIF in human endometrial cancers was statistically significant (Chart 1; $p=1.38E-04$).

We queried a second microarray data to validate differential expression of MIF in endometrial cancer. Again, we observed differential expression of MIF when comparing endometrial tumor tissue to benign endometrial tissue (Chart 2). When sorting each of the genes expressed in endometrial tumor tissue based on significance of change in expression as compared to benign endometrial tissue, MIF ranked 2336 out of 54675 transcripts, equating to 95.7% differential expression (Chart 2). Differential expression of MIF in human endometrial cancers was statistically significant (Chart 2; $p=1.30E-03$).

MIF is expressed at significantly higher levels in endometrial cancers as compared to benign endometrial tissue.

We obtained exact mRNA expression levels for MIF in endometrial tumor tissues and from benign endometrial tissue to evaluate direction and statistical significance of change in expression of MIF in human endometrial cancer. MIF was expressed at higher levels in endometrial tissue as compared to normal endometrial tissue, and this difference was statistically significant (Figure 1; $p<0.0001$). We calculated a mean fold change of 1.14 in MIF mRNA levels in human endometrial cancer, as MIF was expressed at 11.32 ± 0.74 arbitrary units (A.U.) in control endometrial tissue but at 12.95 ± 0.36 A.U. in endometrial tumor tissue.

MIF expression is correlated with patient survival outcomes in endometrial cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between MIF primary tumor expression and survival outcomes in 543 patients with endometrial cancer. We observed a correlation between primary tumor expression of MIF and recurrence-free survival in patients with endometrial cancer, in white patients with low mutational burden, in the upper survival quartile (Figure 2). MIF primary tumor mRNA levels were a positive prognostic indicator in white endometrial cancer patients with low mutational burden. White patients with low mutational burden whose primary tumors expressed low levels of MIF possessed, on average, markedly shorter median RFS as compared to white patients with low mutational burden whose tumors expressed high levels of MIF. This difference in RFS based on MIF tumor expression in white patients with endometrial cancer with low mutational burden approached statistical significance (Figure 2, Chart 3; logrank p -value: 0.055; hazard ratio: 0.26 (0.06-1.14)). MIF primary endometrial tumor expression was not correlated with recurrence-free survival in white patients with high mutational burden (Figure 2, Chart 3; logrank p -value: 0.77; hazard ratio: 1.17 (0.42-3.28)), nor in black patients with high ((Figure 2, Chart 3; logrank p -value: 0.23; hazard ratio: 0 (0-infinity)) or low mutational burden (Figure 2, Chart 3; logrank p -value: 0.67; hazard ratio: 1.42 (0.28-7.08)).

Thus, by mining published microarray data^{5,6} in an unbiased and systematic fashion, we identified macrophage migration inhibitory factor, encoded by MIF, as among the genes whose expression was most different, transcriptome-wide, in the endometrial tumor tissue of patients

1 with endometrial cancer when compared to benign endometrial tissue; we observed
2 significantly increased expression of MIF in endometrial tumor tissue as compared to benign
3 endometrial tissue. Further, we found a correlation between MIF expression and patient
4 survival outcomes in human endometrial cancer, as recurrence-free survival was superior in
5 patients whose tumors expressed higher levels of MIF as compared to patients whose tumors
6 expressed lower levels of MIF, in white patients with low mutational burden, but not in white
7 patients with high mutational burden, nor in black patients with high or low mutational burden.

8 **Discussion**

9 We provided evidence here that macrophage migration inhibitory factor is among the genes
10 most differentially expressed in human endometrial cancer, that mRNA for MIF is present at
11 significantly increased quantity in endometrial tumor tissue as compared to benign endometrium,
12 and that MIF primary tumor expression is correlated with recurrence-free survival in white
13 endometrial cancer patients with low mutational burden. These data suggest MIF may be of
14 importance to fundamental biological processes that underlie the initiation, progression or
15 maintenance of human endometrial cancer.
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Rank: 133
Probe ID: 217871_s_at
p-value: 1.38E-04
t: -5.4641808
B: 1.34597
Gene: MIF
Gene name: macrophage migration inhibitory factor (glycosylation-inhibiting factor)

Chart 1: MIF is differentially expressed in endometrial cancer when comparing primary endometrial tumors to benign endometrial tissue.

The rank of global differential expression, probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Rank: 2336

probe ID: 217871_s_at

p-value: 1.30E-03

t: -3.3102749

B: -1.18862

Gene: MIF

Gene name: macrophage migration inhibitory factor (glycosylation-inhibiting factor)

Chart 2: MIF is differentially expressed in endometrial cancer when comparing primary endometrial tumors to benign endometrial tissue.

The rank of global differential expression, probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Figure 1: MIF is expressed at significantly higher levels in the endometrial tumors of patients with endometrial cancer when compared to benign endometrium.

The mRNA expression level of MIF in benign endometrial tissue (left) and in primary tumors of the endometrium (right) is graphically depicted; the result of a statistical test evaluating significance of difference in MIF expression between benign endometrial tissue and primary tumors of the endometrial tissue is $p=0.0050$.

Figure 2: Correlation between MIF primary tumor expression and recurrence-free survival in endometrial cancer, in white patients with low mutational burden.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival for $n=543$ total endometrial cancer patients stratified into two groups, based on low or high expression of MIF in patient primary tumors, in black patients with high mutational burden (top left), black patients with low mutational burden (top right), white patients with high mutational burden (bottom left), and white patients with low mutational burden (bottom right), in the upper survival quartile. The log rank p -value denoting statistical significance of difference in recurrence-free survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on MIF expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.